THE CONDITIONS of RELEASE of a HISTAMINE-LIKE SUBSTANCE from ISOLATED GUINEA-PIG'S LUNGS.

T H E S I S

Presented to the Faculty of Medicine

of the

University of Edinburgh

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for

the Degree of Ph.D.

1935.

CONTENTS.

INTRODUCTION.

This investigation was started with the object of elucidating the nature of the histamine-like substance (H-substance) recovered from guinea-pigs' lungs during anaphylactic shock, and was then extended to an enquiry into the rôle played by this substance in the anaphylactic reaction. Finally attempts were made to find out whether the liberation of a principle of a similar nature takes place under certain conditions other than anaphylactic shock.

The thesis is mainly concerned with the two latter problems and they will be dealt with in the first section. Contributions towards the study of the identity of the H-substance is mainly contained in two previous communications (de Burgh Daly and Schild, 1934, and de Burgh Daly, Peat and Schild, 1935) enclosed herewith, and the results of the research incorporated in these papers will be referred to briefly in connexion with the present investigation and embodied in the second section which reviews the available information as to the nature of the H-substance.

Historical Review. Theories of Anaphylaxis.

There are two main lines along which an explanation of the phenomena of anaphylaxis has been sought. The theories fall into two groups and may be defined as the humoral and anaphylatoxin hypotheses on one side and as cellular and histamine hypotheses on the other side. This research has a bearing on both of them and they will now be briefly considered.

The anaphylatoxin hypothesis which is mainly associated with the names of Friedemann and Friedberger originated from the observations of Vaughan and Wheeler (1907) that highly poisonous substances can be obtained from cleavage of proteins. Friedemann (1909) later showed that all the symptoms of anaphylaxis can be reproduced in guinea-pigs injected with suitable proportions of antigen and antibody incubated for a short time. The basic assumption of the theory is that of an instantaneous attack on the antigen by a proteolytic enzyme (the complement) activated during the antigen-antibody reaction.

The enzyme was at first supposed to originate from the blood. This view seemed untenable after/

after the demonstration by Schultz (1910) and Dale (1912) that anaphylactic shock can be reproduced in isolated organs. Friedberger and Seidenberg (1927), however, contended that traces of serum remained behind, imbibed into the subcutaneous tissue. Other authors advanced the theory of an intracellular digestion of the antigen. A series of objections of an immunological nature have been raised against this theory, such as absence of latency period. Two objections of a different type should be recorded here. They are both based on experiments with isolated organs and both adduce incompatibilities of time relations against an enzymatic antigen cleavage.

Dale (1912) suggests that although the latency period between the second antigen injection and shock is longer than that of a histamine contraction, the rate of contraction which finally occurs is too quick to be accounted for by the gradual release of a substance. And Bartosch, Feldberg and Nagel (1933), working on isolated guinea-pig's lungs, find that the concentration of the H-substance, released from the lungs into the circulation during anaphylactic shock, does not run parallel to that of the antigen in successive samples of perfusate collected after shock. More direct evidence against the protein/

protein cleavage hypothesis will be brought forward in this paper.

While results obtained on isolated organs do not support the antigen-anaphylatoxin hypothesis, it must be recognised that experiments under these artificial conditions can at the best reproduce one side of the complex phenomena of anaphylaxis. In the whole animal anaphylatoxin formation may be an important factor (H.G. Wells, 1929) and indeed may be responsible for such phenomena as serum sickness.

The cellular theory of anaphylaxis which in its immunological aspects has been mainly developed by Richard Weil (1910) and from a more physiological standpoint by Schultz, Dale, Manwaring, Th. Lewis and Feldberg and their schools, presents two important modifications. They are expounded in an illuminating contrast in Dale's Herter Lecture (1920) and in his Croonian Lecture (1929) respectively. A "change in the dispersion of the protoplasmatic colloids" appeared to be the most economical hypothesis to Dale in 1919 and to Doerr as late as 1928 (Doerr, 1929). The great similarity of the action of histamine to anaphylactic shock had not of course escaped the notice of these authors. Indeed, it is emphasised in the first published communication relating/

relating to histamine as a natural product (Dale and Laidlaw , 1910). But as Dale points out, explaining anaphylaxis in terms of histamine implies that we know more about the action of histamine than that of anaphylaxis and therefore it seems more logical to explain the latter in terms of the former.

The change in outlook was prepared by Manwaring's experiments (Manwaring, Hosepian, Enright and Porter, 1925; Manwaring, Hosepian, O'Neill and Moy, 1925, etc.) showing that the blood from dog's livers after shock contains a histamine-like substance which, as proved by transplantations, is produced in the liver, and further by Th. Lewis's researches on the triple response of human skin. This response was also exhibited in anaphylactic phenomena, which thus were brought into one line with other disturbances produced by their hypothetical H-substance (Lewis and Grant, 1926).

The basis, however, for the histamine theory of anaphylaxis to which Dale adhered in the Croonian Lecture (1929) was the isolation of large quantities of histamine dipricate from ox lungs extracted with the utmost care and speed (Best, Dale, Dudley and Thorpe, 1927). It has been suggested that histamine may not be present in free solution inside the/

the cell but liberated as such "in the very act of cellular death." Lewis's indiffiusible H-substance would lend support to the assumption of a precursor of histamine. Claims for precursors for other hormones (Novadrenine - see Annau, Huszak, Svirbely and Szent-Györgyi, 1932; Euler, 1933; Schild, 1933) have not hitherto been substantiated.

The most decisive contribution to the histamine theory of anaphylaxis has been the work of Bartosch, Feldberg and Nagel (1932, 1933) which in view of its importance for our subject should be reviewed rather more fully.

Feldberg and his co-workers recovered from the Tyrode perfusate of isolated guinea-pig's lungs during anaphylactic shock a substance which in every quality tested was similar to histamine. This fluid ("shock" fluid) contained a substance which contracted the isolated gut, lowered the atropinised cat's blood pressure and released adrenaline from the suprarenals in the same quantitative proportion as histamine. A qualitative similarity was that it contracted the guinea-pig's uterus, but as a rule it failed to relax the rat's uterus, which, however, was also unaffected by equivalent amounts of histamine. On some occasions the rat's uterus was relaxed by the "shock"/

"shock" fluid (perfusion fluid collected during shock), but in those cases the same effect was produced by control perfusates collected before shock. The active fluid contracted the desensitised gut and when injected into normal guinea-pig's lungs it produced a complete respiratory stoppage, thus imitating the effects of shock. Chemically the substance appeared to be stable to 30 minutes boiling and 40-70 per cent. of the activity could be recovered from methyl and ethyl alcohol.

These findings were confirmed and extended by the researches of Daly, Peat and Schild (1935) and Daly and Schild (1934). Using a modified Einthoven technique of respiratory pressure registration, these authors found an actual quantitative agreement within experimental limits between the histamine effect of shock fluid as tested on guinea-pig's gut and cat's blood pressure and its bronchoconstrictor and vasoconstrictor action in guinea-pig's lungs. They further showed that on perfusing the untreated "shock" fluid through the actual lung from which it had been recovered it caused in the desensitised preparation the same bronchoconstriction as its histamine equivalent. This seems to be the nearest approximation to a proof that during shock one or more substances are/

are liberated having powerful pharmacological effects on the actual organ from which they are released. Finally the substance proved to be stable to prolonged boiling in concentrated acid and to be destroyed enzymatically under the same conditions as histamine.

The problem of the significance of histamine in anaphylaxis had been approached before Feldberg from an entirely different angle by Watanabe. (Watanabe, 1931; Watanabe and Hosoya, 1931). This author tested the amount of gut contracting substance that could be extracted from guinea-pig's lungs after sensitisation and before and after shock. He found a more than twenty-fold increase in activity during sensitisation and a corresponding decrease after shock. These experiments were repeated by Daly, Peat and Schild who were unable to confirm Watanabe's claim. They found that the difference in H-substance content of lungs in different stages of sensitisation and shock lay within the limits of experimental error. In parallel estimations where the sampling error was reduced by using batches of three guinea-pigs, the amount of histamine equivalent per g. lung weight in each batch differed by as much as 80 per cent. Notwithstanding Daly, Peat and Schild/

Schild did not deny the possibility of a diminution of H-substance occurring during shock, but they considered that the large variations encountered in normal animals rendered the method for estimating small changes, which might occur as the result of sensitisation or shock, wholly unreliable. They reported that the average amount of H-substance liberated into the perfusate during shock of isolated lungs in 38 ovalbumen sensitised animals was 4.49y, and the average amount of H-substance recovered by extracting 61 lungs, 32.8y per lung. On the basis of control experiments the assay error was ± 25%, thus the loss of H-substance of 5y or less from shocked lungs would not be detectable with any certainty.

However, Bartosch (1935) in a recent communication claims to have discovered a deficit of H-substance after shock by a different method. In perfused lungs he clamped off one lung before injecting the antigen. The histamine content (gut equivalent) per dry weight of tissue was diminished by 10-47 per cent. after shock, while there was no difference if one lung was shocked and the outflow tubing clamped before histamine had time to appear in the perfusate, thus retaining in the lungs the secreted/

secreted H-substance. In control experiments the disparity between right and left lung was less than 5 per cent. The author does not publish the actual figures nor show any tracings*. Until he does so, judgment must be reserved as this is mainly a problem of experimental error.

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* We understand that Dr. Bartosch will shortly publish a detailed account of his experiments.

THE PART PLAYED BY THE H-SUBSTANCE in ANAPHYLACTIC SHOCK

In the preceding section it has been described how the histamine theory of anaphylaxis, which is as old as the discovery of histamine, but which had originally been rejected by cautious observers, has gained in substance during the last ten years. In fact it cannot be denied any more that histamine or a closely allied compound is liberated during anaphylactic shock. This appearance of the H-substance does not, however, prove that histamine itself plays an essential rôle in the production of shock or of its symptomatology. The following research is a contribution to the problem of assessing the part which the H-substance takes in the anaphylactic reaction.

Several important distinctions have been shown to exist in the whole animal between histamine shock and anaphylactic shock. Such are diminished coagulability of the blood, fever and skin necrosis which all occur in anaphylaxis but not with histamine. Dale has explained these discrepancies by assuming that the anaphylactic reaction is produced by three components:

(a)/

(a) histamine set free

(b) other substances liberated (anticoagulant etc.)

(c) direct changes in the cell produced through the antigen antibody reaction.

Manwaring and Marino (1927) have brought forward evidence to show that in the dog at least the symptoms of anaphylactic shock are mainly due to one "shock organ", the liver. Thus he finds that the bladder of a dog into whom the liver of a sensitised animal had been transplanted will contract on injection of the antigen, while a sensitised bladder transplanted into a normal dog will not respond. These experiments seem to indicate that in some organs the released H-substance is mainly responsible for the anaphylactic reaction. Thus, in the guinea-pig, Bartosch, Feldberg and Nagel (1933) state that a complete bronchoconstriction is obtained by re-injecting the H-substance recovered from two shocked lungs into a normal one. It is from this point that our research commences.

The problems examined fall under three main headings:

1. Whether the bronchoconstriction is proportional to the amount of histamine liberated and can be reasonably attributed to this release alone, and whether anaphylactic bronchoconstriction without H-substance release can occur.

2. Whether bronchoconstriction is indispensable for the production of the H-substance.

3. Whether an H-substance release is associated with bronchoconstriction evoked by other agents besides the injection of antigen into sensitised animals.

Experimental Methods.

The methods used in this investigation are essentially the same as those employed by Daly, Peat and Schild (1935) and are fully described in their paper. The description of the methods will therefore be confined here to the essentials necessary for the understanding of the subject matter. For exact detail especially of the mechanical part of the apparatus reference may be sought in the above mentioned paper.

White guinea-pigs from an inbred stock, weighing 250-400 g, fed on oats, bran and cabbage, and kept at a temperature of 67° F. were used. They were sensitised by subcutaneous injections of 10 mg powdered ovalbumen (B.D.H.) and the antigen injected into the isolated perfused lungs 3-12 weeks after sensitisation. They were killed by a blow on the neck and a cannula was inserted into the trachea. The chest wall was then opened and cannulae tied into the pulmonary artery and left auricle.

For perfusion a solution of the following composition is used:

NaCl/

	NaCl	8.0 g
	KCl	0.2
anh.	CaCl	0.1
	MgCl	0.1
	$NaHCO_3$	1.0
	NaH_2PO_4	0.05
	Aq. dest. ad	800.0 ml

This is essentially a glucose-free Tyrode solution in which all the constituents are concentrated in a ratio of 4:5. It will be referred to as T.C.

The perfusate is kept at 36-38° C. by a thermostat. The head of pressure under normal conditions is at 3-5 cm above the level of the arterial cannula. Outflow is recorded by a Harris (1931) drop recorder.

For respiration and registration of the intrapulmonary pressure a modified Einthoven respiratory pump is employed. It consists of a metallic pump system with adjustable stroke and rotating valves for the inflow and outflow of air. At the height of pressure a brief communication with the bromoform manometer system is established which measures the pressure of inflation (respiratory pressure) at constant air volume delivery.

Injections are made into the tube leading into the arterial cannula. For testing the perfusate longitudinal strips of the isolated guinea-pig's gut are/

are used in a Burn and Dale (1922) bath. A constant volume of prewarmed fluid is added to the funnel shaped bath, where it is diluted in a ratio of 1:2.4. In case the perfusate could not be tested immediately after collecting, it was as a rule boiled up for 3 min. in a waterbath to inhibit a possible decomposition by an enzyme.

Relation between Bronchoconstriction in Shock and Histamine Output.

In order to make the subsequent statements more intelligible a brief description of the course of the shock phenomenon in isolated guinea-pig's lungs will be given. Symptoms develop 15-75 sec. after injection of the antigen into the arterial tube. Within 5-10 sec. lobes of the lung cease breathing and usually become quiescent in the full inspiratory position. In some instances, however, a complete expiratory stoppage occurs. This was especially the case if the lungs had been perfused for a prolonged time - one hour - with Ringer fluid before injecting the antigen. The response in those instances was of exceptionally long duration.

Vasoconstriction usually develops together with the bronchial tube response or slightly later, it progresses after the bronchoconstriction has reached its maximum and generally subsides before the bronchial effect. The perfusion pressure as a rule requires to be considerably raised (from 3-4 cm often to 14-15 cm) in order to drive the perfusate through the vascular system in shock. In the majority of experiments during the fall of respiratory pressure all the lobes of the lungs do not collapse equally/

equally and one or more appear over-distended.

The H-substance content of the first sample corresponds approximately to 1 in ½ million of histamine (gut assay), and in the subsequent samples sometimes rapidly drops to very low activities (1:100 million and less); it may, however, remain high 1 hour or more at concentrations up to 1 in 16.6 million. The total output varies between 0.0 and 12y, the average, as mentioned before for ovalbumen experiments being 4.49y. Graph I (page 48) illustrates the course of some typical shock experiments.

It will be seen from this description that the effects of shock are in most cases supramaximal and irreversible and do not lend themselves to a quantitative estimation.

One exceptional case, however, in these experiments is of special interest and worth noting. That is those experiments in which no output of histamine was detected, which means that 5-10 cc of perfusate collected during shock had an activity of less than 1:100-1:200 million. In two of those experiments the animals were sensitised with horse serum. In both cases the bronchoconstriction was not maximal and of short duration. The most interesting/

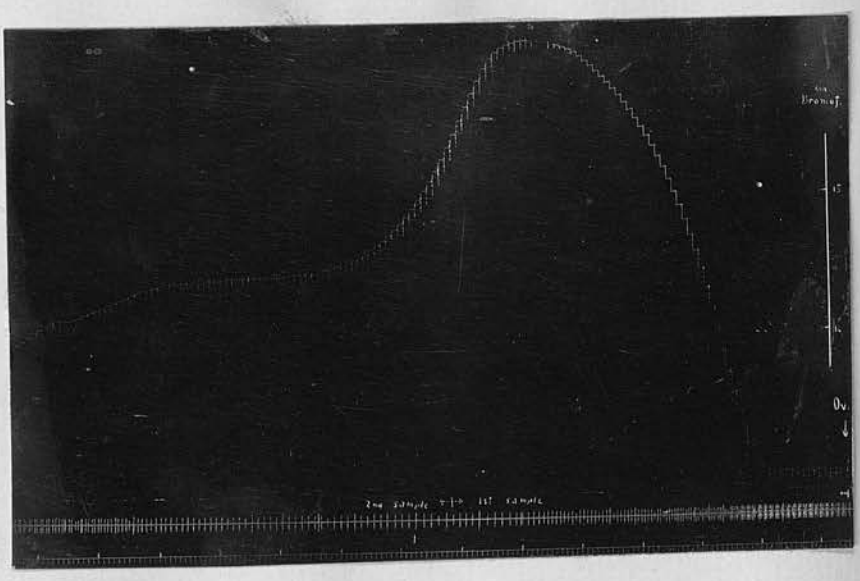


Fig. 1. Guinea-pig's perfused lungs. Respiratory pressure and pulmonary venous outflow. (Also in all other figures). On injection of 10 mg ovalbumen short lasting complete bronchoconstriction. No H-substance was detected in the perfusate. Read from right to left.

interesting case is that of an ovalbumen-sensitised guinea-pig which was shocked after a prolonged perfusion.

Experiment (Fig. 1)

In this experiment the antigen was not injected in the usual manner but the perfusion fluid was changed to one containing $\frac{1}{2}$ mg ovalb./cc. After a relatively long latency period of 80 sec. - the dead space effect could account for 20 sec. only - a complete bronchoconstriction and a moderate vasoconstriction occurred. The bronchoconstriction subsided quickly. The first sample of shock fluid (11 cc) and the second (17 cc) were inactive, both containing less than 1:100 million H-substance. This means that less than 0.1ly H-substance was liberated during the period of maximal bronchoconstriction. The perfusates in these cases were not tested immediately but kept on ice overnight. We have never observed a loss of activity on standing. The histamine solution against which the perfusate was standardised was made up with the same ovalbumen concentration.

We have not been able to repeat this experiment, which is the only case of a complete bronchoconstriction during shock without H-substance release/

release recorded amongst over 50 perfusions. The three experiments quoted are marked out from all the other experiments by the weakness of their bronchial response. It would appear, therefore, that the H-substance is almost invariably released if a prolonged maximal bronchoconstriction occurs during shock.

Atropine

Introduction: The aim of experiments with atropine was twofold. In the first instance to decrease the anaphylactic reaction to such an extent that it should become comparable with injected histamine; in the second instance to see whether histamine would be released with lungs completely relaxed on antigen injection.

If the bronchoconstriction and vasoconstriction produced in normal lungs by perfusion with "shock" fluid or with histamine is quantitatively comparable with these effects occuring on perfusion of the antigen through a sensitised lung, atropine should diminish the shock response to the same extent as it diminishes the histamine response. Again, if a gross discrepancy occurred, the question arises whether this can be adequately explained by the different mechanism of release of the H-substance or whether the anaphylactic response is determined by further factors in addition to the H-substance.

Literature: The following scattered data were found in the literature regarding the effect of atropine on histamine and on shock in guinea-pigs. They all/

all refer to experiments in the whole animal.

Dale and Laidlaw (1910) report that 5 mg. atropine increased the lethal dose of histamine from 0.5 to 1.0 mg. and La Barre (1926) finds a similar shift of the lethal dose of histamine from 0.35 mg. to 1.0 mg. by a preventive injection of 1.0 mg. atropine/100 g. Injecting the atropine after histamine, Dale and Laidlaw could not relax a bronchoconstriction produced by 0.5 mg. histamine with 5 mg. atropine, and Koessler and Lewis (1927) state that 0.5 mg. atropine does not release the bronchoconstriction after 5.0y histamine. Regarding effects on the anaphylactic bronchoconstriction Auer and Lewis (1910) were able to save 3 out of 5 guines-pigs from death by injecting 3.0 mg. atropine before shock and Mita (1911) states that 2.0 mg. atropine injected intravenously will delay, but not prevent, anaphylactic death. Hanzlik and Karsner (1920) prevented death in shock by a prophylactic injection of 1.0 mg. atropine/100 g. but found marked changes in the guinea-pig's lungs on autopsy. With 0.02 mg./100 g. atropine Leyton and Leyton (1916) obtained no effect on the symptoms of shock.

Experimental: In a first series of experiments/

experiments we have investigated the effect on histamine contractions of different amounts of atropine added to the perfusion fluid. The relatively low effectiveness of atropine on histamine is well known and is confirmed by our experiments on guinea-pig's lungs. The following concentrations of atropine sulphate are needed to reduce a histamine contraction to a liminal one:

5y atr/cc	for	0.2y hist.	
25y		1-2y	
200y		5-10y	(3 experiments)
1000y		100-200y	(3 experiments)

It is necessary to make the comparisons on completely relaxed lungs. As soon as progressive bronchoconstriction sets in, sensitivity to histamine decreases considerably. On further increasing the concentration of atropine progressive bronchoconstriction occurred and for this reason such experiments were not pursued. The shock reaction in a sensitised lung is not visibly altered by a content of 25y or 200y/cc atropine in the perfusate and is quite marked though diminished in size and duration with 1.0 mg/cc. A single injection of 5.0 mg atropine was ineffective in preventing shock if the antigen was injected 20 minutes later.

The/

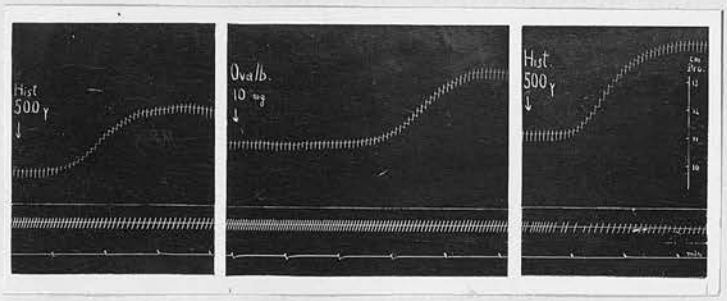


Fig 2. The perfusion fluid contains 1 mg atropine/cc. Subsequent injections at about 15 minutes interval. Anaphylactic shock equals 500 y histamine.

The shock reaction thus appears to be definitely stronger than that due to an injection of 100-200γ histamine when the perfusate contains atropine.

This figure agrees with the result of another set of experiments in which an attempt was made to match the shock contraction in the atropinised preparation with the contraction due to histamine injections. In 1.0 mg./cc atropine perfusions, the vascular and bronchial effect occurring during shock was equivalent to doses of from 300-1000γ histamine (Fig. 2). In these experiments the histamine acid phosphate was injected in 0.1 cc. of fluid into the arterial cannula tube: the histamine was carefully neutralised. As an additional control an equivalent amount of sodium acid phosphate was added to the ovalbumen injected.

Is the H-substance more atropine resistant than histamine? One interpretation of the fact that the secreted H-substance appears more atropine resistant than histamine, is that an additional factor enhances the action of the H-substance in the presence of atropine. In this connection the perfusate has a low surface tension and this might potentiate the action of the H-substance in the presence of atropine. Moreover strong atropine solutions have a marked surface/

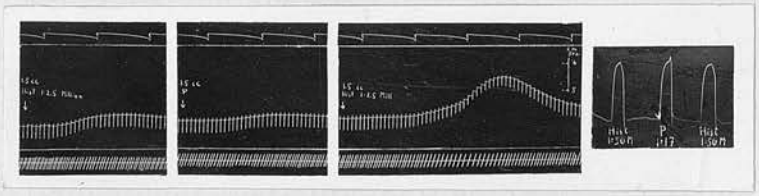


Fig. 3. Tracing on the right: isolated guinea-pig's gut (Magnus). Perfusion fluid contains 5y atropine/cc. A "shock fluid" (P) which on the gut has an activity equivalent to 0.33y histamine/cc is less active than 0.4y/cc on the lungs.

surface activity, for in concentrations of 1:1000 Tyrode solution there is considerable foaming when air is passed through the fluid. Thus in those cases where atropine exerts its antagonistic effects in high concentration only, it might act by blocking the surface of the cell, just as any other surface active substance would do. In this event the surface active H-substance might be expected to displace atropine from the cell surface and therefore neutralise it to a greater extent than would pure histamine.

Experiment:

H-substance recovered from a "shocked" lung was compared against histamine on a lung perfused with 5y/cc atropine. Contrary to expectation its action was found to be slightly diminished, as compared with its gut equivalent of histamine. (Fig. 3).

A similar experiment was performed on the gut. It was known from previous experiments that small doses of atropine, which do not affect the histamine contraction, have no effect on the H-substance response of the gut. The action of large doses of atropine has not been tried.

Experiment/

Experiment:

A sufficient quantity of atropine sulphate was added to the bath just to abolish the action of a given dose of histamine. It was found that 10y/cc just abolished the effect of 5.0 cc histamine 1:50 million, added to a 7.0 cc Tyrode fluid containing bath. The same dose of atropine abolished the effect of a gut equivalent of the H-substance.

It appears from these experiments that neither on the lung nor on the gut is the H-substance more atropine resistant than histamine. Whether the small discrepancy in the opposite direction found in testing the H-substance on the atropinised lung is significant has not been investigated.

Effect of atropine on liberation of H-substance:

The fact that the contractile response of the gut to histamine is inhibited by high doses of atropine limited the atropine concentration which could be adopted in these experiments, and, therefore, the crucial experiment of testing the "shock" fluid collected from lungs completely paralysed by atropine could not be performed. But it was found that the addition of 25y/cc atropine to the perfusion fluid

neither/

neither decreased the shock response nor the H-substance release; in one experiment 5y H-substance was recovered.

Discussion: The dose of 500y histamine necessary to produce effects equal to shock in the atropinised preparation is very high in relation to the amount of H-substance liberated during shock (5-10y) and to that present in the lungs (50-100y), even taking into account the differences in action between a released and an injected substance. The difference in the site of action is not so fundamental in this case as it is if the release of a substance by nerve stimulation and its injection are compared. Both the antigen and histamine are introduced through the same route and should produce their effects on the same cells. This also applies to the atropine. There remains however the distinction between the action of substances on the outside of the cell surface on the one hand released from the cell itself and on the other presented directly to the external surface of the cell, the importance of which cannot be estimated.

No definite conclusion can be drawn, therefore, from these experiments as to whether the H-substance alone can account for the smooth muscle contraction in shock. But they appear to us to favour the assumption that/

that an additional factor which is not secreted with the H-substance may be acting in shock.

An interesting side result of these experiments is the finding that doses of atropine necessary for abolishing the gut effects of histamine are of the same order of magnitude as those which antagonise the bronchial histamine action. This disposes of the theories that histamine on the lungs has a peculiar affinity for the parasympathetic system. It also is an illustration that in drug antagonism the quantitative aspect is as important as the qualitative one.

Adrenaline

Introduction: Experiments with atropine had failed to provide information on the problem whether a bronchial inhibitor drug would also inhibit the H-substance output, because of the difficulties met with in testing on the gut the atropine-containing perfusate. The same difficulty applies to most inhibitor drugs. Probably no substance which inhibits bronchoconstriction is without effect on the guinea-pig's gut. This latter effect must be eliminated from the perfusate before it can be assayed on the gut for its histamine-like activity. The treatment to bring about such elimination must fulfil two conditions. It must not affect the activity of the H-substance present and it must completely inactivate the inhibitor drug. In fact the main problem of the following experiments is that whether a drug which counteracts shock will also diminish or abolish the liberation of H-substance. It follows that the presence of minute quantities of H-substance must be detectable without interference by the inhibitor drug.

Adrenaline being a relatively easily inactivated substance seemed to be most appropriate for the kind/

kind of experiment. The subject to be discussed in this section of connection between inhibition of symptoms of shock and inhibition of H-substance output illustrates the new alignment in the problems of anaphylaxis through introduction of the notion of an H-substance. The question up to now was whether a drug which prevents smooth muscle contraction also inhibits the antigen-antibody reaction. The criterion for the occurence of an antigen-antibody reaction when the smooth muscle contraction in shock is inhibited is a subsequent desensitisation. Apparently a complete desensitisation by a single injection is more easily obtainable in some tissues than in others, and hence some discrepancies in interpretation have arisen. On the isolated guinea-pigs uterus Dale (1913) obtained complete desensitisation after a single injection. He finds that if the first shock contraction is inhibited by hypertonic solutions and the uterus is then put back into an isotonic solution where it is fully irritable, desensitisation has occurred. No contraction follows a second antigen injection.

Different conclusions are arrived at by Elkeles (1928) who, using guinea-pig's gut with chloralose as an inhibitor agent, in analagous experiments to those of Dale, finds the gut still irritable

on/

on a second injection of antigen. He, therefore, thinks that the anaesthetic has inhibited the antigen-antibody reaction. Meyer (1929), however, points out that the conclusion is not justified, as guinea-pig's gut will fully respond to several doses of antigen if the latter is washed out between injections. The same conclusion can be drawn from Kendall and Shumate's (1930) experiments who succeeded in producing nine successive antigen contractions in guinea-pig's gut, if the bath fluid was replaced by fresh fluid after each contraction.

In this connection some experiments by Berger and Mutsaars (1934) from Doerr's Institute are of interest. These authors report that if antigen is added in alcoholic solution to the isolated sensitised gut no contraction occurs, but the contraction takes place if the alcohol is washed out and replaced by water. They interpret these experiments, without direct proof, as an inhibition by alcohol of the egression from the cell of an active substance, followed by a removal of the inhibition when the alcohol is displaced by Ringer. It may be concluded that, although dissociation between inhibition of antigen-antibody reaction and of shock symptoms has not been proved

with/

with certainty to occur in all cases, it does take place in some instances (Dale 1913) as we shall confirm. The antigen-antibody reaction, therefore, is not of necessity linked to smooth muscle contraction.

The question arises: Is the histamine output connected with one of the two phenomena - antigen-antibody reaction and smooth muscle response - or with both, or with a third independent factor? Experiments with adrenaline should throw some further light upon the problem.

Literature: Adrenaline has generally been found to counteract bronchoconstriction by histamine or anaphylaxis. Baer and Pick (1913) report a return of respiration in isolated lungs if a 10y/cc histamine perfusate was supplemented by one containing 10y/cc adrenaline. Koessler and Lewis (1927) obtained a release of the bronchoconstriction due to shock and to histamine in whole animals by 0.5 mg adrenaline. Henzlik and Karsner (1920) point out the importance of injecting adrenaline (5y/100 g) simultaneously with the antigen.

Experimental part:

Destruction of adrenaline: As a preliminary a method had to be devised to destroy the adrenaline present/

present in perfusates before testing them. All that was required for our purpose was to eliminate any activity of adrenaline on the histamine contraction of guinea-pig's gut. This factor then was the only one tested and controlled in our experiments. Without quoting the huge amount of literature on the destruction of adrenaline (Sugawara 1928, Wiltshire 1931, Barker, Eastland and Evers 1932, Schild 1933) a short account of the methods which we found suitable and unsuitable for our purpose will be given.

The adrenaline used in these experiments was the crystalline B D.H. preparation made up with 1cc $\frac{N}{10}$ H Cl/10 mg.

Method I. Adrenaline 1:50,000 and histamine 1:50 millions in Tyrode solution (pH~8.2) are boiled for 10-30 minutes in the water bath with oxygen. The solution is then tested on guinea-pig's gut against one containing the same amount of histamine but no adrenaline. The treated solution appears to be only half as active as the control. It is concluded that boiling in Tyrode fluid for 30 minutes did not completely inactivate the adrenaline present.

Method II.

(a)/

(a) Method I. is modified by adding alkali to the fluid to produce a final concentration of 0, 2N Na OH. The solution after treatment is found to be on the gut fully active and it is concluded that boiling in 0.2N Na OH has inactivated the adrenaline present.

(b) The same dose of histamine, adrenaline and Na OH is dissolved in Tyrode which had been previously perfused through the lungs of a guinea-pig. After subjecting the solution to the same treatment as in the former cases it had no action on the guinea-pig's gut. It is concluded that the method is not suitable for our purpose as either the adrenaline is not inactivated or else the histamine is destroyed under these conditions.

(c) To test the last assumption experiment II(b) was repeated without the addition of adrenaline. After treating the solution most of the activity had again disappeared. It is concluded that boiling in an alkaline Tyrode fluid which had been previously perfused through guinea pig's lungs has inactivated histamine. (See also Best, Dale, Dudley and Thorpe, 1927).

Method III. 200y/cc adrenaline are added to a histamine/

histamine containing Tyrode or perfusate or to H-substance. A clean scraped copperfoil is immersed into the fluid and air is bubbled through for an hour at 40° to 50°. The fluid gradually becomes markedly red and then pale brown. In every case this solution is as active as an adrenaline free control.

Conclusion: By using copper as a catalyst (Barker, Eastland and Evers, 1933) adrenaline can be inactivated under conditions which leave the H-substance unaffected.

Preparation of Adrenaline: A further technical difficulty which caused much trouble and seems worth mentioning was the preparation of adrenaline. 10 mg adrenaline base was dissolved in 0.5 cc $\frac{N}{5}$ H Cl. To this solution shortly before it was to be used an equal volume of either $\frac{N}{5}$ or $\frac{4N}{5}$ $NaHCO_3$ was added. In the first case sufficient CO_2 was left in the solution to make it definitely acid, in the second case the pH was approximately equal to that of Tyrode fluid. Even when a four times equimolar dose of $NaHCO_3$ was added the adrenaline did not seem to undergo oxydation in the short period until/

until it was injected. But if this procedure was adopted at higher concentrations of adrenaline such as 50 mg/cc it tended to precipitate out suddenly on stirring.

A satisfactory solvent for concentrated solutions of adrenaline is boric acid (50 mg adrenaline in 1 cc of 2.5% boric acid) which yields a practically neutral solution (Höchster Farbwerke 1905).

Although the point was not investigated systematically it seems likely that the salt and acidity of adrenaline injected may play an important part in the response and some indications supporting this will be given in the next section.

Effect of various ways of administering adrenaline: To achieve the object of the experiments, viz. a complete suppression of bronchoconstriction in shock different ways of administering adrenaline were tested:

1. Perfusion with adrenaline at 10 y/cc and 100 y/cc had but little effect in suppresing the shock bronchoconstriction. A complete bronchoconstriction was obtained on antigen injection, the adrenaline effect manifesting itself only as a shorter duration/

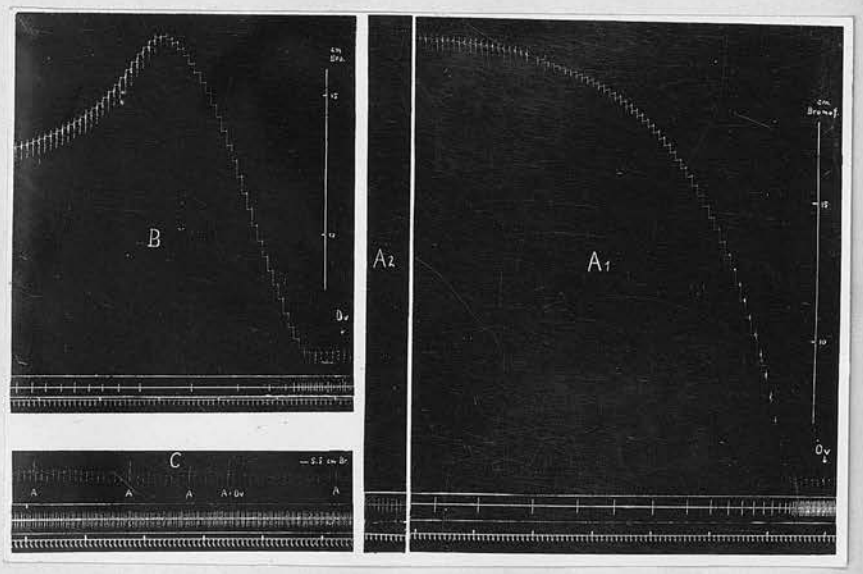


Fig. 4. A1: Anaphylactic shock in control. A2: same tracing 12 minutes later. B: anaphylactic shock with 100y/cc adrenaline in the perfusate. C: injection of ovalbumine simultaneously with adrenaline. Read from right to left.

duration of the constriction (fig 4). The negative result was probably not due to destruction of adrenaline in the thermostat, as the outflowing fluid was but faintly pink in contrast with the deep red which developed when adrenaline was destroyed.

2. Next, 1-3 injections of 100 y to 5 mg adrenaline before injection of the antigen were given. The last one preceded the antigen by one minute. Bronchoconstriction was less, one half to two-thirds of maximum.

3. The following method of injection was then tried: 0.1cc of solution containing 1 mg of adrenaline were injected three times into the arterial tube, the first dose three minutes before the antigen, the second dose together with it, the third dose one minute after it. (TABLE I). Of four experiments, two were highly effective in suppressing bronchoconstriction, two were partly effective only. In these experiments the adrenaline chloride was neutralised with $\frac{N}{10}$ $NaHCO_3$ solution.

4. The same procedure as 3 was adopted in two other experiments in which adrenaline was neutralised with a $\frac{4N}{10}$ $NaHCO_3$ solution. In both cases marked, though not complete, inhibition of bronchoconstriction was/

TABLE I.

EFFECT of ADRENALINE on BRONCHOCONSTRICTION and LIBERATION of H-SUBSTANCE in SHOCK Isolated Perfused Lungs					
Batch	Ovalbumen	Adrenaline	Bronchial Effect	Adrenaline Experiment H-Substance Output	Control Experiment H-Substance Output
A	10 mg injected into artery	Adr. neutralised with $\frac{N}{10}$ Na HCO_3 3 x 1 mg	Very slight	0.935y	3.41y
A	"	"	Slight	0.4y	1.42y
B	"	"	$\frac{1}{3}$ maximal	1.55y	2.32y
B	"	"	$\frac{1}{2} - \frac{2}{3}$ maximal	3.68y	3.77y
C	"	Adr. neutralised with $\frac{4N}{10}$ Na HCO_3 3 x 1 mg injected	$\frac{1}{5} - \frac{1}{4}$ maximal	1.225y	6.35y
C	"	"	$\frac{1}{4}$ maximal	1.92y	>5.45
C	0.5 mg Ov/cc sol perfused	" but 4 x 1 mg injected	Absent	0.455y	6.17y
C	"	"	Absent	0.5y	1.57y

was obtained. (TABLE I).

5. 5 mg adrenaline in boric acid was injected three times in the same manner. In spite of the five-fold dose of adrenaline a marked effect of shock occurred.

6. In all the previous experiments 10 mg ovalbumen was injected in 0.1 cc of Tyrode fluid into the arterial tube. In this series ovalbumen was perfused through by changing over the perfusion to a solution containing 0.5 mg ovalbumen /cc. 1 mg adrenaline was injected four times in roughly two minutes' intervals, the first dose being given two minutes before the change. (fig. 4). This procedure in two experiments completely inhibited any bronchial effect. (TABLE I)

As to vascular effects it could not be ascertained whether they are abolished, as they are superimposed on those of adrenaline, which always produces a small vasoconstriction (Dale and Narayana, 1935).

Output of H-substance. The results concerning output of H-substance in these experiments are summarised in Table I.

Each experiment was controlled in the following/

following way. A guinea-pig of the same batch and approximately the same weight was perfused usually on the same day. In every case an equal amount of adrenaline was added to the shock perfusate of the control, and this was subjected to exactly the same treatment (Method III) as the adrenaline perfusate. This precaution was taken although previous experiments had shown that no destruction of H-substance occurs under these conditions. Both extracts then were tested against histamine on one piece of gut.

The Table comprises the experiments under headings 3, 4 and 6 of the previous chapter. In the other cases, where a test for histamine was made controls are lacking. It is of interest, however, that perfusion with 100 y/cc yielded the average output of 3.35 y.

The following points relating to Table I. should be noted:

1. In all cases, except two, the histamine output in the control was at least three times larger than in the adrenaline experiment.

2. The two exceptions were also characterised by relative ineffectiveness of adrenaline on bronchoconstriction.

3/

3. Though experiments are too few, they seem to indicate that the effects of adrenaline on bronchoconstriction are greater if the antigen is perfused instead of injected in a single dose, and if the adrenaline is alkaline rather than acid; the same indications apply to the inhibition of H-substance output.

4. In no case was the H-substance output completely abolished.

Desensitisation. It would seem possible that the inhibition of H-substance output by adrenaline is due to incompleteness of the antigen-antibody reaction. If this is the case the tissue should not be completely desensitised and a second injection of antigen after the adrenaline effect has passed off should cause bronchoconstriction.

To test this assumption we have in two cases reinjected the antigen 20-30 minutes after the first dose of ovalbumen, the bronchoconstrictor action of which had been inhibited by adrenaline. The second dose of antigen had no effect. This lack of smooth muscle response was not due to a persistence of the adrenaline action, as 1.0y of histamine caused a small but definite response.

It is concluded that adrenaline does not impair the antigen-antibody reaction and that the diminished/

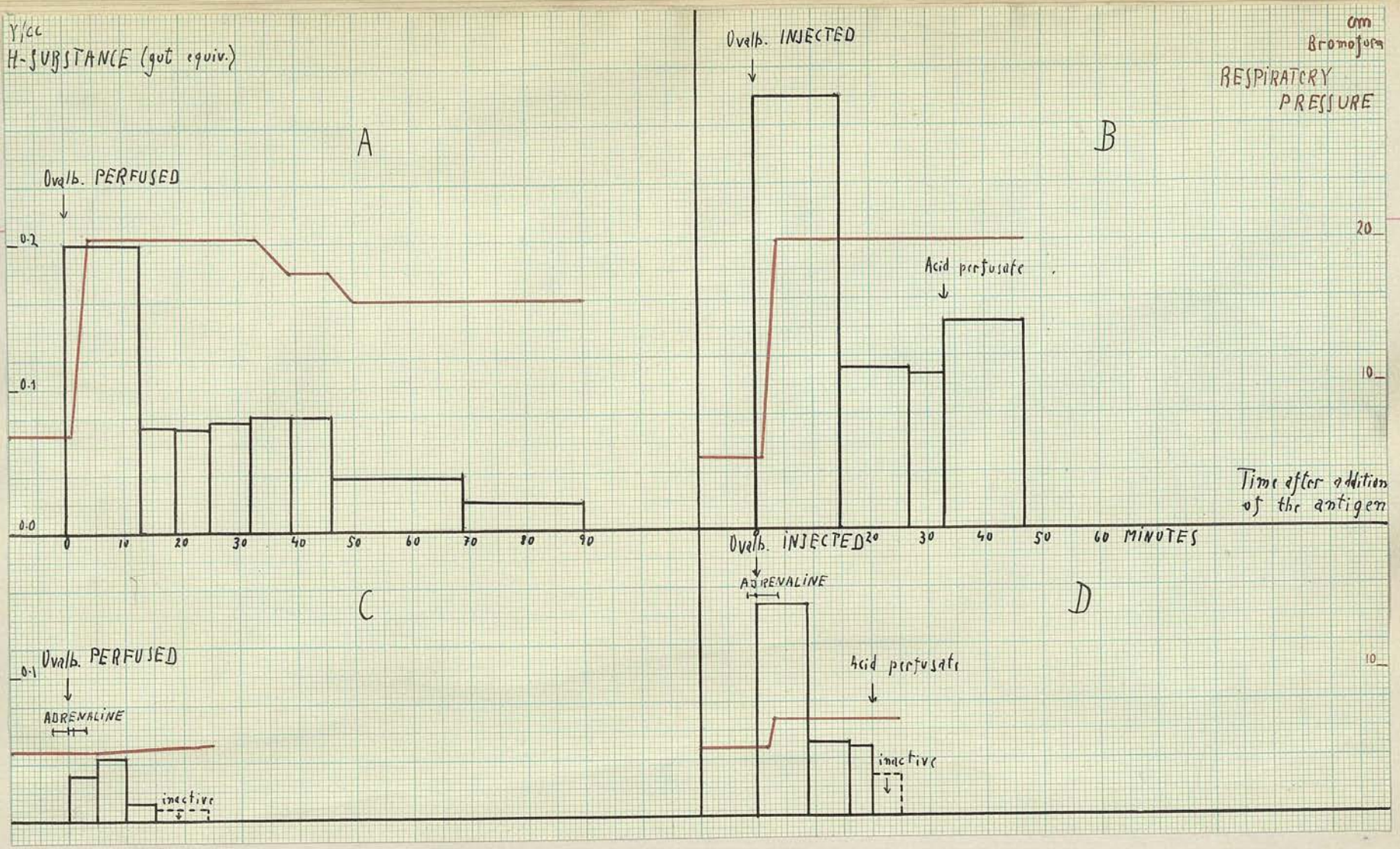
diminished output cannot be due to its incompleteness.

Time Relations of Output. There is one variation between the experiments with adrenaline and the controls, which might explain a discrepancy in the output of H-substance and which had to be eliminated. When ovalbumen alone is injected into a sensitised lung a powerful vasoconstriction occurs. The vascular effect is much feebler if adrenaline is given simultaneously with the antigen. It follows that in the control experiments the ovalbumen is in a more prolonged contact with the cell. The longer time available for the antigen-antibody reaction might result in a greater H-substance output.

To test this hypothesis an ovalbumen solution of constant concentration was perfused through the lungs instead of injecting the antigen. Thus the concentration and the duration of contact of the antigen with the cell was made independent of the flow. Adrenaline in this case was just as effective in reducing the amount of liberated H-substance as when the antigen was injected (TABLE I).

These experiments are of interest in connection with the problem of time relations of output/

output. It has been shown previously by Daly, Peat and Schild (1935) that the liberation of H-substance is not instantaneous but prolonged. It was demonstrated that the continued appearance of activity in samples collected after shock is due to a persistent formation of H-substance and cannot be satisfactorily explained by a simple "wash out effect". Our experiments with adrenaline and with perfusion of the antigen seem to indicate that this prolonged output is established at the moment of the clash of the antigen with the cell and that it is not influenced by later events. Indeed if this were not so, it should be expected that the concentration of H-substance would rise as the adrenaline effect passed off to figures comparable to those obtained in control experiments. This should happen especially in the case of ovalbumen perfusion, where the supply of antigen is continuous. In fact the disappearance of the adrenaline action has no noticeable effect on the liberation of H-substance and the general shape of the time-output curves with adrenaline is on a smaller scale identical with that of controls. To illustrate these relations four characteristic experiments with injection and perfusion of ovalbumen, both with and without adrenaline are/



GRAPH I. Histamine-like activity of subsequent samples of perfusion fluid from isolated guinea-pigs' lungs after shock. Respiratory pressure (red). Showing (1) that there is no essential difference between the effects of a single injection and a perfusion of the antigen, (2) that the presence of adrenaline when the antigen-antibody reaction first occurs diminishes the output of H-substance also of the subsequent samples (3) that acid does not inhibit liberation of H-substance after shock (B).

are presented in GRAPH I.

It is concluded that although there is a prolonged production of H-substance the course of events is governed by the condition of the tissues at the time when the antigen comes in contact with the sensitised tissue.

Discussion. During shock there is a marked decrease of H-substance output if adrenaline is present. This is independent of the duration of time the tissue is in contact with the antigen and after shock there is complete desensitisation. If it is assumed that the quantity of antibody is constant and that the phenomenon of desensitisation means that all the antibody is used up, then the effect of adrenaline in reducing H-substance output suggests that H-substance formation and antibody destruction are not wholly dependent upon one another. Is the amount of H-substance liberated related to the degree of bronchoconstriction? A relation of this kind certainly exists but only over a limited range, it seems to fail at both end points. A 50% reduction of bronchoconstriction is not associated with a noticeable decrease in output, while apparent complete inhibition of bronchoconstriction by adrenaline does not completely abolish H-substance output. Both these peculiarities/

peculiarities may be apparent and due to technical limitations. Obviously a small statistical discrepancy in output cannot be detected by such few experiments. Again, an inherent limitation of our method of measuring bronchial pressure is the fact that a constriction accompanied by a compensatory dilatation in another area is not registered. It is thus not definitely established by these experiments whether the diminution of H-substance output when adrenaline is present is connected with the inhibition of smooth muscle response or with another more specific action of adrenaline.

RIGIDITY of the LUNGS and H-SUBSTANCE.

Introduction: The previous experiments seemed to indicate that bronchoconstriction itself is a factor influencing the output of the H-substance. At this juncture two questions naturally arise: Does rigidity of the lungs, as such, cause liberation of histamine? Does "injury" cause liberation of the H-substance in the lungs? The first problem is relatively new, and has hitherto only been dealt with by Bartosch, Feldberg and Nagel (1933). They produced a bronchoconstriction by injecting histamine into lungs. There was no increase of H-substance in the perfusate. The second problem has caused a great deal of speculation especially in connection with the problems of traumatic shock and of skin injury.

The question of histamine liberation by tissue injury which was first raised by Bayliss and Cannon (1918) cannot be discussed at length here. Recent observers, however, seem to agree that no direct experimental proof of an H-substance present in the blood from injured regions has been brought forward. This also applies to the H-substance formed in skin injuries, whose presence has not hitherto been directly demonstrated (Lewis and Grant, 1924, Percival & Scott 1931)

There/

There is, however, a very great body of elaborate indirect evidence worked out mainly by Sir Th. Lewis and his School, which supports the claim that a histamine-like substance is produced by injuries of the skin. It will be sufficient here to refer to the triple response and to symptoms such as fall in blood pressure and gastric secretion produced in patients with urticaria facticia by extensive scratching of the skin.

It has been the aim of these experiments to investigate whether several agents which cause rigidity of the guinea-pig's lungs will also produce a liberation of H-substance. We have again been limited in the choice of such agents by the same considerations as set forth previously. Their activity should not interfere with the test on the guinea-pig's gut.

Hitherto the effects of varying the pH of distilled water and of K ions have been investigated.

Distilled Water.

The effect of distilled water on plain muscle has been systematically investigated by Meigs (1912, 1914, 1915) and by Underhill under Lovatt Evans (1926). Both these authors report an increase in weight which is more gradual than with striated muscle, but goes on for a more prolonged time and reaches higher values. There is another important difference between striated and plain muscle when immersed in distilled water. The former simultaneously with the production of lactic acid contracts strongly, the latter, while it swells up, does not shorten to any extent (Underhill l c). Trendelenburg (1912) examined the action of distilled water on the isolated bronchi of oxen. An initial contraction followed by complete relaxation occurred.

We were led to experimenting with distilled water by the observation that hypotonic solutions produce a gradual increase in respiratory pressure (Daly, Peat and Schild, 1935) without the appearance of the H-substance in the perfusate. It seemed likely that distilled water should cause a more pronounced rigidity and conceivable that it should be accompanied by H-substance release.

Experimental/

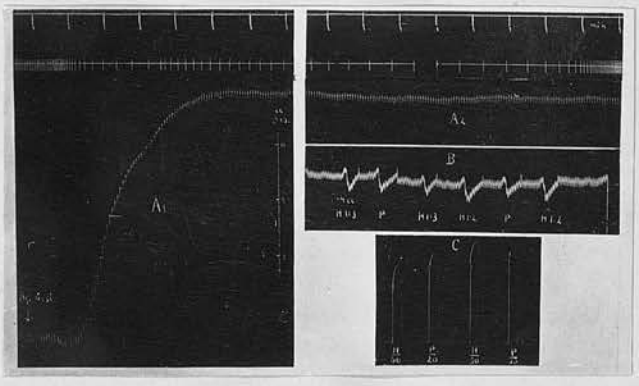


Fig. 5. A2 same tracing as A1, 14 minutes later. B: atropinised cat's blood pressure under ether. C: longitudinal strip of guinea-pig's gut. Distilled water perfused through lungs. Sample of perfusate is equivalent to 1:2.75 million histamine on the gut and to 1:2.5 million histamine on the cat's blood pressure.

Experimental Part.

Experiment I. (fig. 5).

In a non-sensitised guinea-pig the perfusion is changed from T.C. (Control Sample I) to aq. dest. After one minute a complete bronchoconstriction and an extremely strong vasoconstriction occurs. The perfusate is collected (Sample II) and then the perfusion is changed back to T.C. The bronchoconstriction persists, but an intense vasodilatation sets in (Sample III). A further sample is collected (Sample IV).

Test		Guinea-pig	Cat's blood pressure
I.	17 cc	Inactive 1:200 million	
II.	4.2 cc	1:275 million	1:2.5 million
III.	16.5 cc	1:10 million	
IV	11 cc	1:25 million	

Experiment II.

A sensitised guinea-pig is perfused with T.C. and then the perfusion fluid changed to aq. dest. Intense broncho- and vasoconstriction resulted. A small quantity of perfusate is collected (Sample I) and 10 mg ovalbumen injected into the arterial tube. No obvious change of respiratory pressure occurred. A further sample is collected (Sample II). Perfusion is changed back to T.C. (Sample III).

Test/

Test on guinea-pig's gut:

Sample	I	2.5 cc	1:15 million
Sample	II	4.8 cc	3: 5 million
Sample	III	32 cc	1:11 million

Result: Distilled water perfused through lungs liberates a substance which quantitatively agrees with histamine on the guinea-pig's gut and on the cat's blood pressure. The substance is produced both in the sensitised and the unsensitised animal. Injection of antigen into a sensitised lung perfused with distilled water causes a further increase in the activity of the perfusate.

Discussion: There is a superficial resemblance between the reactions to a perfusion with distilled water in the normal or sensitised lung and those to injection of antigen in the sensitised lung. Nothing certain, however, can be concluded as to the similarity of the intrinsic mechanism of the response. In fact, in both cases it is not established to what extent the rise in respiratory pressure is due to an active bronchoconstriction and how far to a passive swelling and occlusion of the bronchial tubes. Analogy with other plain muscle suggests that actual water-clogging/

waterclogging of the tissue plays an important part in the rigidity observed. The complete irreversibility of bronchial response would also suggest a similar explanation were it not that the vascular effects are completely reversible.

If we try to analyse the nature of the injury which the tissue might endure through the distilled water, again very little can be said on the base of the existing evidence. If the plain muscle tissue behaved osmotically like a semipermeable system, as for instance the red blood corpuscles, it should lose its semipermeable qualities in distilled water. Meigs (l c) however in his experiments on frog's ventricle and on the clam Venus Mercenaria comes to the conclusion that plain muscle does not behave as if surrounded by a semipermeable membrane when immersed in distilled water, but that it swells up continously like a gel. In conformity he finds that it remains irritable even after the uptake of large amounts of water.

It appears, therefore, that it cannot be definitely stated whether distilled water causes the liberation of H-substance by so completely destroying the cell structure that all its contents will leak out, or/

or rather by a more specific action which will cause a liberation of H-substance in a certain analogy to anaphylactic shock.

Potassium Chloride

Trendelenburg (1912) states that KCl moderately contracts isolated ox bronchi. Our experiments prove the same in regard to isolated guinea-pig's lungs. Moderate contraction of bronchi and vessels was obtained if the perfusate was changed to one containing a tenfold concentration of KCl; a sudden marked constriction occurred if 0.1 cc of a 20% KCl solution was injected (20% Na Cl is ineffective). A rapid constriction also takes place if KCl free perfusate is introduced at the beginning or during a perfusion.

The respiratory pressure curve remained low for prolonged periods with perfusates containing as little as 0.12 gm/lit and as much as 0.55 gm/lit KCl. The lungs in these cases respond readily to shock which causes the liberation of large quantities of H-substance.

The perfusates obtained during bronchoconstriction through lack of potassium or excess of potassium were tested on guinea-pig's gut. The tests were negative. The limit of sensitivity in these gut tests was lower than usual, at about 1:10 million. This is due to the fact that perfusates had to be diluted until their potassium con-

tent/

content did not interfere with the histamine contraction. Equal amounts of KCl were added to the histamine test solutions.

Result: Lack of potassium and increase of potassium produce bronchoconstriction. No detectable amount of H-substance is liberated by K ions or by lack of potassium.

Variations in pH.

The effect of pH on plain muscle has been reviewed by Lovatt Evans (1926) who came to the following conclusions:

1. Acidity in general relaxes, alkalinity contracts plain muscle.

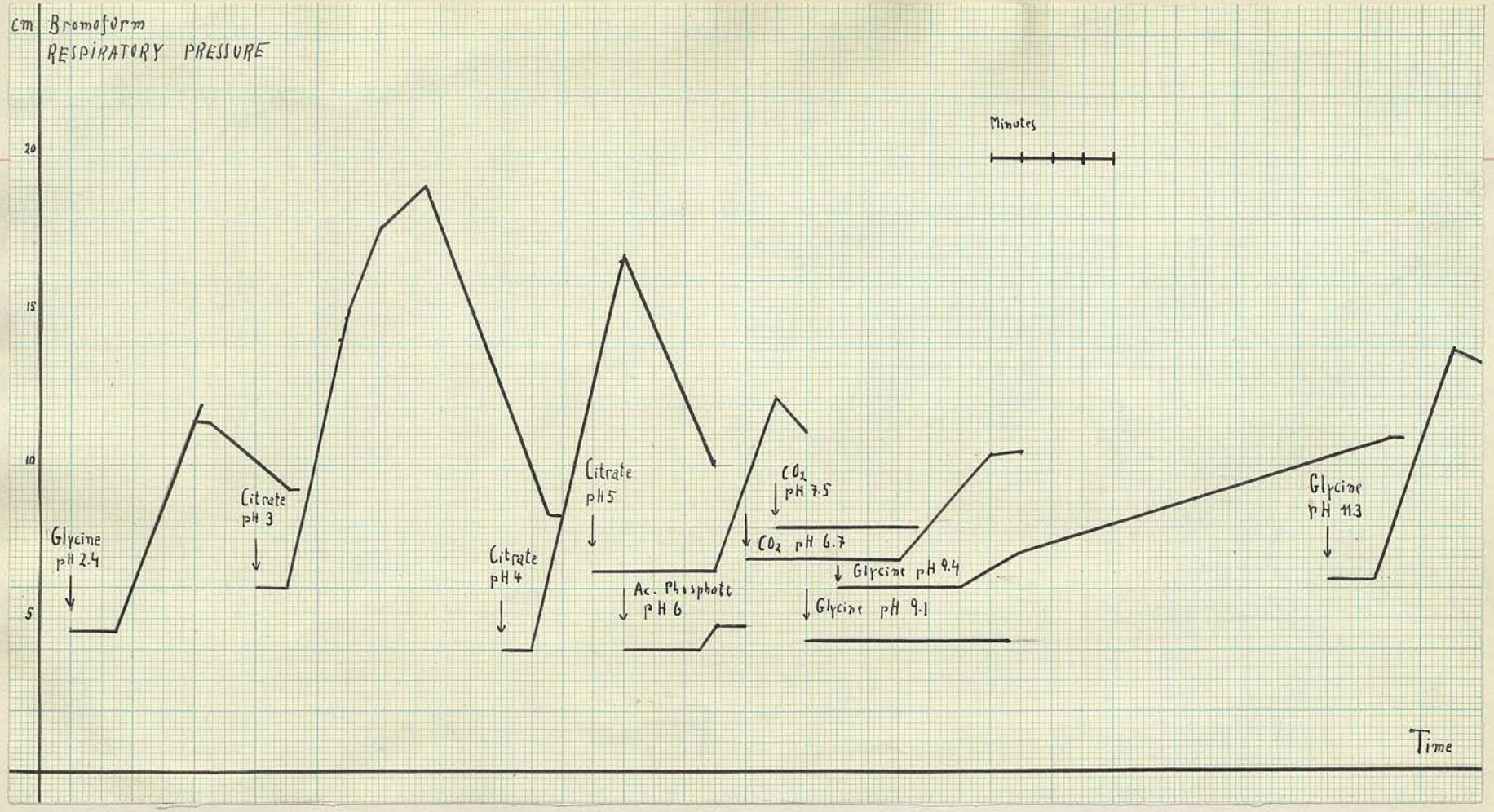
2. At a pH outside the range 4-10 a rapid swelling of the tissue sets in, which according to Lovatt Evans can be explained as an imbibition of water.

On the lung Trendelenburg (1912) found a dilator action of acid and a constrictor one of alkali when working on isolated ox bronchial rings. Thornton (1931) with the tracheal perfusion method of McDowall and Thornton (1930) and using Van Dyke and Hastings' (1927) Ringer obtained a contraction on changing the perfusate from pH 7.0 to pH 7.8 and a relaxation on returning to the initial fluid.

We have investigated changes of pH over a big range. Our object was to find out whether along with the effects on the respiratory pressure and on the vascular system a liberation of H-substance would occur/

TABLE II.

ISOLATED LUNGS PERFUSED with BUFFERED SOLUTIONS of DIFFERENT pH.					
Whether Sensitised or Normal	Buffer Used	Approximate pH	Flow Effects	Effect of Several agents introduced after the pH change	H-Substance Output.
S	HCl glycine 7 : 3	2.4	Marked	10 mg ov ineffective 10y histamine ineffective	inactive < 0.1y
N	HCl citrate 7 : 3	3	Moderate	100 hist. ineffective return to T.C. causes broncho- & vasoconstriction	inactive same as control
N	HCl citrate 6 : 4	4	Very slight	2 y hist. ineffective T.C. marked flow eff. slight bronchoconstr.	
N	Citrate	5	none	With T.C. marked R P & flow effects	citrate inactive<1:50 mil. T.C.<inactive 1.50 mil.
N	1 cc conc. HCl to 1000 cc T.C. with phosph.bicarb.	6	Very slight		inactive << 1:50 million
N	CO_2 bubbling	6.7	slight	2y hist. strong effect	
N	CO_2 bubbling	7.5	absent	T.C. no effect 0,2y hist. good effect	
N	Glycine - Na OH 7 : 3	9. 1	small	1y hist. ineffective 50y hist. effective	
N	Glycine - Na OH 5 : 5	9.4	marked		< 0.1y
N	Glycine - Na OH 2 : 8	11.3	very strong		inactive << 0.3y



GRAPH II. Isolated perfused guinea-pig's lungs. Effect on respiratory pressure of perfusion fluids at varying H ion concentrations.

occur. In a second series of experiments instead of perfusing solutions of a known pH a small amount of acid was injected into the arterial tube.

Experimental Part.

Determination of pH. The buffers employed were standard buffers according to Clark (1928). The actual type and amount of buffer employed is recorded with the experiment, unless otherwise stated, the final concentration of all buffers was $\frac{N}{100}$ in a phosphate and bicarbonate free T.C. The loss of Na ions was adequately compensated for. The final solutions were compared colorimetrically with standard buffer solutions according to Clark. The figures given do not pretend to be exact pH values, but their indicator colours are always 'bracketed' between a slightly more acid and a slightly more alkaline solution.

Results: The results on bronchoconstriction of a change to perfusates of different pH are recorded on Graph II. Further points regarding these experiments are summarised in Table II. It will be seen from this data that:-

1. Guinea-pigs lungs do not respond by broncho-

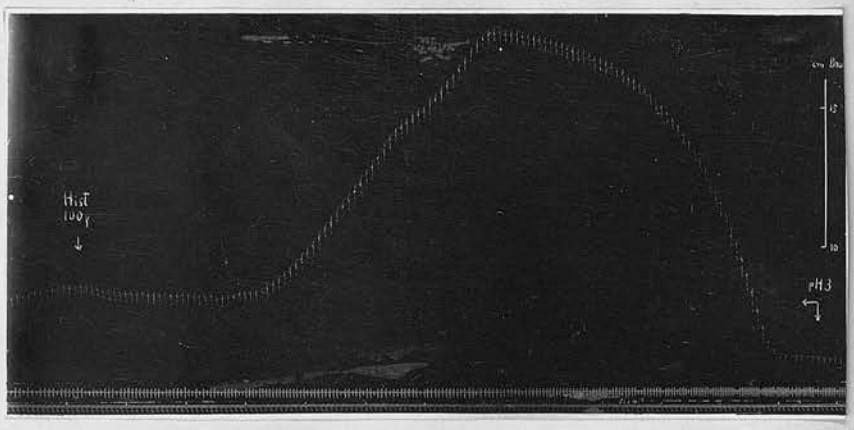


Fig. 6. Effect of acid perfusion fluid (pH3). 100γ histamine after acid ineffective.

bronchoconstriction to relatively wide changes in pH. Alkaline solutions have no effect at pH 9, but an effect gradually appears at a higher pH, but bronchoconstriction is incomplete even at pH 11.3.

A change from T.C. which has a pH of 8.2 to a solution of pH 7.5 is ineffective. With a lower pH a constrictor effect of acid appears. With CO_2 buffer a moderate constriction at pH 6.7 occurs, phosphate buffer at pH 6 is practically ineffective, and even at a pH 5 constriction is incomplete and the latent period is long (5 minutes). It is only at a lower pH than this that a sudden maximal bronchoconstriction takes place which always gives way to a relaxation later on (fig. 6).

The rôle of the Na ions in buffers has not been investigated separately, but the effects recorded (with exception perhaps of CO_2) seem to alter directly with the H ion concentration, independently of the buffer used. Citrate in these concentrations did not percipitate the Ca from Tyrode which agrees with the data given for solubility of calcium citrate.

2. Both in acid and in alkali bronchoconstriction is accompanied by vasoconstriction but flow effects are much weaker in relation to bronchial effects than/

than in alkaline solutions. Flow in acid tends to return to normal.

3. No output of H-substance could be detected from sensitised or from unsensitised lungs.

Before testing perfusion solutions on guinea-pigs' gut they had to be carefully neutralised, which was relatively easy as the amount of acid or alkali present was known. Neutralised control Ringer with histamine was effective, while without histamine it had no action. In confirmation of Lovatt Evans and Underhill (1923) we found acid inhibiting and alkali contracting guinea-pigs' gut.

4. At pH 9 although the lungs were completely relaxed sensitivity to histamine seemed diminished. After strong acid, though the respiratory pressure may fall nearly to the original level, no effect occurs with big doses (100 y) of histamine or by injection of the antigen in the sensitised animal. In these cases, however, a marked constrictor response is always obtained by changing back the perfusion to the original T.C.

Effect of Acid Perfusion after Shock. Perfusion with acid solutions had caused complete bronchoconstriction, but no output of H-substance. This might/

might have been due to the fact that the acidity of the perfusate counteracted the release of the H-substance. In the following experiments this assumption was tested.

Two sensitised guinea-pigs were "shocked" and the perfusate was collected. 30 to 40 minutes after shock, perfusion was changed to an acid perfusate of pH 5 respectively 4. In both cases flow increased while concentration of the perfusate remained unaltered (1:10,000,000) or rose (1:12 to 1:10 million). Under normal conditions with an increase in flow the concentration falls, while here increase in output per unit time has occurred. (GRAPH I)

Result. Profusion of an acid Ringer solution after shock does not inhibit the liberation of the H-substance.

Injection of Acid. Small amounts (0.1 cc) of HCl were injected straight into the arterial tube. $\frac{N}{10}$ $\frac{N}{2}$ $\frac{N}{1}$ solutions were employed.

0.1 cc $\frac{N}{10}$ in the sensitised and the unsensitised guinea-pig causes no bronchoconstriction and no H-substance output.

0.1 cc $\frac{N}{2}$ HCl on first injection produced a strong but not maximal bronchoconstriction and a liberation of 0.33y. A second injection in the constricted/

constricted preparation caused no further broncho-constriction and a liberation of 0.96y H-substance.

0.1 cc $\frac{N}{1}$ HCl in another normal guinea-pig had similar effects with liberation of 0.91y H-substance.

Result. H-substance is liberated in the sensitised and the unsensitised animal by injecting small amounts of dilute HCl.

Discussion. The main result of these experiments is that from lungs perfused with Ringer solution within ranges of pH from 2.4 to 11.3 no H-substance is liberated. This absence of H-substance is the more interesting as there is a complete and sudden rigidity of the lung and undoubtedly considerable injury. The experiments disprove the assumption that a complete rigidity of the lung will cause in itself liberation of H-substance. It may be pointed out, however, that the respiratory stoppage was never very prolonged. Injection of a very small amount of diluted HCl did cause liberation of H-substance. The effective pH in these cases was probably lower than in perfusions and it is conceivable that even very short contact may cause a real disintegration of cells. Another possible interpretation/

interpretation is that a sudden injection may have a qualitatively different effect from a gradual perfusion.

The effects recorded on bronchoconstriction are of interest but their significance is limited by the following considerations:

1. All the effects are obtained on a tissue which had been previously immersed in a solution of pH 8.2. Under these conditions irritability may be altered and a response be elicited which is not obtained under normal conditions. As an instance of an effect which is solely due to an abnormal starting condition it may be mentioned that a bronchoconstriction is regularly obtained if a calcium-free perfusate is supplemented by one containing Ca, even if the Ca content of the latter is lower than that of the usual Tyrode solutions. (Unpublished observations.)

2. As has been emphasised before a rise in respiratory pressure does not differentiate between active bronchoconstriction and passive obstruction of the bronchial tubes.

In spite of these limitations it is of interest to compare the reactions to changes in pH of plain/

plain muscle from the lungs with that of plain muscle of other sources.

The outstanding difference seems to be the relative insensitiveness of bronchi against alkaline reaction. The gut is very sensitive to alkali (Lovatt Evans & Underhill, 1923; Hammett, 1921; Kupaloff, 1924), which was confirmed when assaying alkaline perfusates on the gut.

The effect of acid, particularly at high H ion concentrations may be one of imbibition of water only as Lovatt Evans suggests. It is difficult to reconcile with this assumption the fact that the respiratory pressure falls abruptly while the acid is still acting. Also the quickness of the response suggests rather a true bronchoconstriction. This is not without parallel in other plain muscle. Thus Fränkel and Morita (1924) in accordance with Lovatt Evans found that dilute HCl relaxes the uterus and the gut in the guinea-pig, but it contracts these organs in the rat.

The strong vasoconstriction by alkali corresponds to that generally found in the systemic circulation. (Literature in Heymann 1921). There is considerable divergence of opinion regarding the effect of acid on perfused vessels (Gaskell, 1880, Bayliss/

Bayliss 1901, Pearce 1913, Heymann 1921, Fleisch 1918, Atzler & Lehmann 1921, etc.). In our experiments vasoconstriction with acid was so slight and transient that it is suggested that it may be merely a secondary effect of the bronchoconstriction. (Dale and Narayana, 1935).

General Discussion.

The problem we set out to investigate in this section was whether lung rigidity and injury would cause a liberation of H-substance. It may be concluded that these conceptions are too indefinite for our problem and that both with lung rigidity and with injury the H-substance is in some cases liberated and in others not. Two agents which caused rigidity of the lung, excess of K ions and perfusion with acid and alkaline solutions, did not liberate H-substance, two other agents, distilled water and small doses of pure acid did produce it. Our experiments do not give a clear indication as to which precisely is the type of injury that will free the active principle. It may be noted, however, that the cases with the more prolonged and intense broncho-constriction (distilled water) and the probably greater injury (injected HCl) were the more effective ones. Unfortunately the most powerful broncho and vasoconstrictors, like Ba ions, cannot be tested because of their action on the gut.

If we turn to the mechanism of release of the H-substance the most interesting fact in connection with our experiments is perhaps the negative results with potassium. It has been shown recently

by/

by Brown and Feldberg (1935) that K ions liberate an acetyl-choline-like substance when injected into the superior cervical ganglion, or into the submaxillary gland (Feldberg, unpublished observation). If H-substance is not liberated by K ions it must be kept inside the cell by a different mechanism. It would seem most profitable at the moment to collect more facts about the liberation of H-substance before attempting a theory on the mechanism of production. This would seem particularly desirable as it is as yet completely unknown whether the H-substance plays any part in contractile, vasomotor or nervous processes in the lung.

CONCLUSIONS.

The starting point of this investigation has been the problem whether H-substance is an essential or an accidental feature in anaphylactic shock.

The following facts have been established:

1. Liberation of H-substance has hitherto been found invariably connected with a strong anaphylactic reaction and a parallelism between the two phenomena is suggested.

2. Atropine in quantitative experiments inhibits the histamine contraction very much more than the shock contraction, if the liberated histamine is taken as a standard of reference.

3. Adrenaline inhibits the liberation of H-substance in proportion as it inhibits bronchoconstriction.

4. Some agents which cause lung rigidity, like distilled water or diluted HCl when injected, liberate H-substance. Other "injurious" agents as perfusion with buffered ringers of pH 2.4-11.3 or K ion produce lung rigidity but no H-substance output.

These/

These results are not sufficiently conclusive to supply an answer to the problem whether the H-substance is the cause or the effect of the smooth muscle contraction in anaphylactic shock.

THE ORIGIN and NATURE of the H-SUBSTANCE.

Before proceeding to the discussion of the problem of the identity of the H-substance a simple experiment bearing upon this point will be described. The object of this experiment is to rule out the antigen as source of the H-substance. It is based on three assumptions: that the H-substance is histamine, that histamine can only be produced from histidine and that no other substance contributes to the gut-contracting activity of the shock perfusate. Under these terms, if the amount of "histamine" liberated during shock is greater than the total amount of histidine injected as antigen, then the "histamine" cannot have originated from the antigen.

The histidine content of ovalbumen has been estimated by various authors. Their figures are summarised in the following table, after H.O. Calvery; (1931):

Koessler and Hanke	2.30%
Kossel and Patten	1.71%
H.O. Calvery	2.44%

It will be seen that each figure is below 2.5%.

The next question is how far the shocking dose of histidine can be reduced. Wells (1929) states that 50-100y ovalbumen is the minimal shocking dose in/

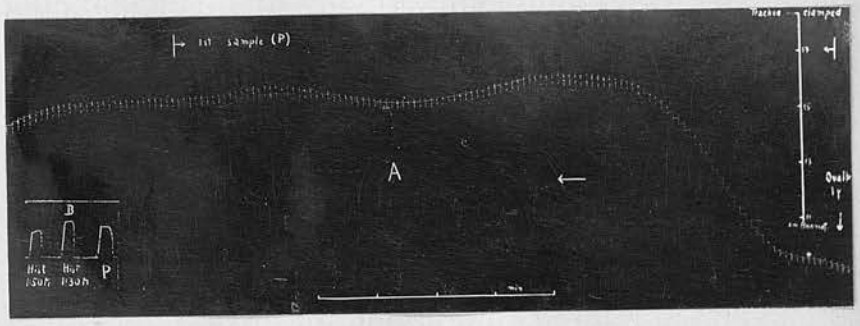


Fig. 7. A: Anaphylactic shock with ly ovalbumen. Incomplete bronchoconstriction.
B: Guinea-pig's gut. Activity of perfusate equivalent to histamine 1: 40 millions.

in the whole animal. We had previously found that no diminution of H-output occurs if the antigen dose is reduced from the usual 10 to 1 mg.

Experimental Part. The effects of 1 and 25y ovalbumen were tried on two guinea-pigs from a well sensitised batch. The average H-output of the batch was 4.41y.

Experiment I

Male, 300 g sensitised 54 days previously with 10 mg ovalbumen. On injection of 25y ovalbumen, complete bronchoconstriction took place lasting 15 min., then gradually subsiding bronchial relaxation occurred.

Assay of perfusate on guinea-pig gut:

Control: negative

Sample I: 5.7 cc 1:7 million histamine equivalent

Sample II: 13.2 cc 1:150 million " "

Total 0.9y " "

Experiment II (Fig. 7)

Male, 280 g sensitised 55 days previously with 10 mg ovalbumen. On injection of 1y ovalbumen 3/4 complete bronchoconstriction lasting 12 min. occurred, then gave way to gradual relaxation.

Output/

Output:

Sample I: 6 cc 1:40 million histamine equivalent

Sample II: negative 1:200 million " "

Total 0.15y " "

Discussion. The maximum amount of histamine derived from the 2.5% histidine contained in ovalbumen could only be 0.44y in the first experiment and 0.018y in the second. These figures have been exceeded roughly by 100% and by 700%. This is with the above mentioned limitations a direct proof that neither humoral nor intracellular digestion of the shocking dose of antigen can have produced all the recovered "histamine".

It will be convenient at this juncture to discuss the evidence of the nature of the H-substance. By H-substance is meant that component of the shock perfusate which causes its main physiological activity. There is direct and indirect evidence for the identity of the H-substance with histamine. Direct evidence is based on the fact that perfusates from shocked lungs behave physiologically like histamine in all respects hitherto tested.

Quantitative agreement was found on the cat's blood pressure, guinea-pig's gut and cat's suprarenal/

suprarenal secretion (Feldberg), again on the cat's blood pressure, guinea-pig's gut and guinea-pig's bronchial and pulmonary vascular effects. Some discrepancies on the rat's uterus can be satisfactorily explained by the concentration of histamine in the "shock" fluid being too low to give the relaxation response, and Dale in fact adduces this negative result as a further proof for the identity of the two substances. Again, the atropine experiments have shown that, while a more atropine-resistant agent might take part in the anaphylactic reaction, the actual perfusate is not more atropine-resistant than histamine.

Of physico-chemical properties tested, histamine and H-substance are equally resistant to boiling and to boiling in concentrated acids. H-substance is destroyed by histaminase and is soluble in methyl- and ethyl-alcohol, although the percentage recovered by us from ethyl-alcohol was slightly smaller than that of pure histamine treated in the same way. There is thus no evidence against the identity of H-substance and histamine, whilst there is strong evidence in favour.

Indirect evidence is based largely on the assumption that the H-substance released in shock, the/

the histamine-like substance extracted from guinea-pigs' lungs, the crystalline product isolated from ox lungs by Dale and co-workers and histamine are identical.

Evidence for the identity of the "shock substance" with that extracted from guinea-pigs' lungs would be strengthened if it could be proved that the H-substance does not originate from the antigen. This proof has been attempted by three independent methods previously described. They are suggestive but none of them is conclusive, Feldberg's because it is too indirect, Bartosch's for technical reasons discussed above and ours because it presupposes the identity of H-substance and histamine thus leading into a vicious circle.

We have shown that by perfusion with distilled water or by injection of a small amount of acid a substance is liberated into the perfusate which has the same properties on blood pressure and gut as the H-substance. It seems reasonable to believe that this active substance may not be different from that obtained by acid extraction (Best) of the whole lung.

A direct comparison of the histamine-like substance/

substance from guinea-pigs' lungs with that from ox lungs has never to our knowledge been made. There would seem a priori to be no reason to doubt the identity of "histamines" in closely related species - just as other hormones with well defined actions have been found distributed and acting in heterogenous species. Such variations as the appearance of creatine phosphoric acid in vertebrates to take up the function of arginine phosphoric acid in avertebrates or as the Fe and Mg in chlorophyll and haemoglobin molecules seems to be associated with the big partitions in biology only.

There has, however, arisen a notion of a species "histamine" mainly through the work of Grant and Jones (1929) on frog skin. These authors found that extracts from ox lung and human skin behaved differently from extracts of frog skin when subjected to a Kossel-Kutscher fractionation. While in the mammalian extract all the depressor activity on the atropinised cat could be recovered in the lysine-arginine fraction, in the frog only one-fifth of this activity was recovered. Moreover, the same extract had a powerful action on the guinea-pig's uterus which was in one case three times, in another fifteen times, greater than that of an equidepressor histamine/

histamine solution. But it is not clearly proved that this fraction in contrast to the control has a histamine-like activity on the frog. A flare on the frog's tongue was produced once out of two occasions, and it appears from the data that a fall in frogs' blood pressure was only obtained by injecting the arginine fraction in a three respectively fourfold concentration of the first extract, while in the mammalian control equi-concentrated solutions were compared.

It is concluded that though these authors have discovered an interesting substance in frogs' skin - which might possibly be related to the arginine bufotoxine present in toads' skin (Wieland and Alles 1922; Flury 1917) - and have shown that such skin contains either very little or no histamine, there is no reason to call this substance histamine-like.

The simpler alcohol soluble constituents of mammalian tissue which have hitherto been specified, such as acetyl choline, adenylic acid, the unidentified substance of Euler and Gaddum (1931) or Major and Webber (1929, 1930, 1932), all present profound physico-chemical and physiological differences from histamine. On the other hand from a tissue-like horse/

horse intestine which contains at least four of the known depressor substances by suitable manipulations, a physiologically active agent can be prepared which will quantatively agree if tested against histamine on three or more different preparations, (Gaddum and Schild, 1934).

Against the identification with histamine of Best, Dale, Dudley and Thorpe's crystalls little criticism has been levelled. Burchard (1934) asserts that the mixed melting point is not conclusive in this particular case, as it is not sharp and suggests that several histamine salts should be crystallised.

Of the other simpler natural bases guanidine and methyl-guanidine have been made responsible for anaphylactic shock by Heyde (1912). Loewit (1913) has shown, however, that it does not cause an emphysema of the lungs in guinea-pigs. There exist, however, a host of higher molecular degradation products of proteins like peptones, protamines, etc. or such substances as crotaline, the venom of rattlesnames (Essex and Markowitz, 1930) which in many ways reproduce the effects of anaphylactic shock and of histamine. In this connection two observations are pertinent.

Gerard/

Gerard (1922) found that intestinal extracts which had been freed of histamine developed new histamine after prolonged boiling in strong acid. He argues that alcohol insoluble protamines may contribute to the histamine-like activity of extracts which would not lose activity on hydrolysis as histamine would be formed by the disintegration of the higher molecular compounds. The author has not, however, excluded from his experiments the possibility of a formation of histamine from histidine which may occur under such conditions (Ewins and Pyman, 1911).

An interesting suggestion is made by Arai (1923). This author finds that esterified amino-acids become active probably through "covering" of the carboxyl group. Thus histidine eythl ester has an action like histamine, but to about a 1000 times lesser degree. Arai suggests that an amino-acid may become active through acquiring ester-like coupling between carboxyl and hydroxyl groups instead of the acid-amide linkage (see also Mitchell and Hamilton, 1929).

That such substances, which seem to be the natural sources of histamine, should take part in the anaphylactic reaction does not seem unreasonable. It could explain why relatively a great part of the H-substance/

H-substance is alcohol insoluble, as the compounds mentioned above are insoluble in alcohol. It would be of interest to test the effect of H-substance on the coagulability of blood which is decreased by shock and by "peptones" but not by histamine. An important feature of these peptone-like substances, as also of crotaline, is not present in the H-substance. The latter remains equally potent in successive injections, thus not producing an immunity against its own action.

Finally, it should be considered briefly whether there is evidence for other physiologically active substances being present in the shock perfusate. On the evidence of Feldberg's and our experiments there is no need to assume the production during shock of another agent besides histamine. The inhibitor action on the rat's uterus and the effect of admixed blood had both been noticed in the perfusate before and after shock and cannot be attributed to it. In every case examined, activity on the atropinised and unatropinised guinea-pigs' gut and on the atropinised and unatropinised cats' blood pressure agreed within experimental limits. This is in contrast with the experiments with the H-substance extracted from whole lungs where as described/

described by Daly, Peat and Schild (1935), in some cases and not in others a significant discrepancy between gut and cat values arose. This can be best explained by the presence of a further gut contracting or histamine enhancing substance, which can be extracted from lungs, under conditions not yet specified, but which is not produced during the reaction.

In summing up it may be said that direct and indirect evidence favours the view that H-substance and histamine are identical.

SUMMARY.

1. The theories of anaphylaxis and evidence for the liberation of a histamine-like substance in anaphylactic shock are reviewed. The properties of the histamine-like substance (H-substance) recovered from the perfusate of guinea-pigs' lungs after shock are described.

2. Experiments are performed on the isolated lungs of sensitised and unsensitised guinea-pigs perfused with Tyrode solution and inflated with a constant air volume. The respiratory pressure and the flow are registered. Perfusates are tested on the guinea-pigs' gut for a histamine-like activity.

3. Some experiments are described where anaphylactic bronchoconstriction without the liberation of H-substance has occurred. The relative weakness of symptoms in these cases supports the hypothesis that a connection exists between a strong shock response and the liberation of H-substance.

4. A quantitative comparison is made of the inhibitor action of atropine on bronchoconstriction from shock and from histamine injections. It is found/

found that histamine is equally effective to shock in the atropinised lung if about a hundredfold of the dose liberated during shock is injected intra-arterially. The discrepancy is not due to a difference between the secreted H-substance and histamine.

5. Adrenaline partially inhibits the liberation of H-substance. This inhibition seems to bear a relationship to the suppression of bronchoconstriction.

6. Distilled water causes symptoms superficially resembling those of shock and a substance indistinguishable from H-substance is liberated.

7. K ion and lack of K ion both produce bronchoconstriction. No H-substance is detected in the perfusate.

8. Buffered Ringer solutions of varying H ion concentrations in a range of 2.4-11.3 were perfused through the lungs. They do not cause liberation of the H-substance. Gradually increasing effects on respiratory pressure rise are obtained with high and low H ion concentrations. The flow is diminished to a greater extent by alkali than by acid.

Injections/

Injections of a small quantity of diluted H Cl cause bronchoconstriction and a liberation of H-substance.

9. It is found that more "histamine" may be liberated in shock than could be accounted for by the histidine content of the antigen.

10. The evidence for the identity of H-substance and histamine is discussed.

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